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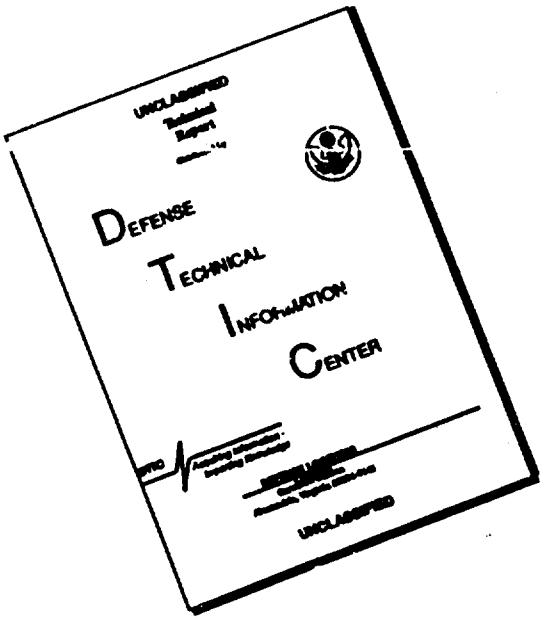

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A LABORATORY INFECTION WITH THE SPOROTRICHUM DE BEURMANNI

Centralblatt für Bakteriologie
und Parasitologie
(Central Journal of Bacteriology
and Parasitology)
Vol. 55 pages 361-370 [see Note 1]
1910

H. Fielitz

Since the discovery of sporotrichosis by B.R. Schenck and its thorough study by de Beurmann this disease has been repeatedly reported on in America, but especially frequently and down to very recently in France, while the few observations in other countries have remained scattered up to the present. The first case of sporotrichosis in Germany, which was observed toward the end of last year at the Royal University Polyclinic for Skin Diseases and Venereal Diseases at Berlin [2] has not been followed by a second, although several articles concerning sporotrichosis have appeared in the German literature and the attention of large numbers among us has thus been directed toward this interesting and practically important disease.

[Note 1] I have already reported briefly on this infection in the Berlin Medical Society on 4 May of this year.

[Note 2] Arndt, G., "A Contribution to Knowledge of Sporotrichosis of the Skin, with Special Attention to Lymphangitis Sporotrichosica. Experimental Sporotrichosis," Dermatologische Zeitschrift (Dermatological Journal), Vol 17, No 1 and 3.

It thus appears that the infrequency of observations on this subject is due not only to the extraordinary difficulty of diagnosis (confusion with tuberculosis and lues!) but rather to the uneven distribution of this disease. Indeed, in our own polyclinic, in spite of the vast field of observation and the fact that our attention was particularly directed toward it, no new observation has yet been made.

The case on which I am reporting in this article does not represent a genuine sporotrichosis, but a laboratory infection which I incurred myself, probably in inoculation of animals or in dissecting animals diseased with sporotrichosis or perhaps in sporoagglutination experiments.

The strain of the fungus which was used in these experiments was derived from the above-mentioned case, and my infection like the other ran the course of the regionally restricted sporotrichosis, but did not reach the same stage of advanced development (lymphangitis covering almost the entire length of the arm, with multiple abscesses), because its nature was correctly identified soon after it set in and it was appropriately treated.

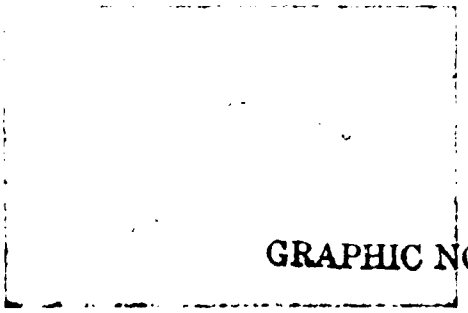
The only skin injury that I am aware of and that could both from the time of its occurrence and from its location come into question as the place of entry of the virus was one that I inflicted on myself on 23 December 1909 for the purpose of an inoculation in experimental work with mollusc material. Before the inoculation the skin on the flexor side of the right forearm, approximately in the middle, in an area where the sporotrichotic inoculation focus later developed, was rubbed with pumice, so that at the place most affected a vesicular dermatitis developed, which disappeared without a trace within two weeks under treatment with powder and sealing bandages.

If this skin injury was causally connected with the infection, the first clinical symptom, which appeared early in March of this year, must have been preceded by an incubation period of about two months.

The disorder first manifested itself in a barely lentil-sized red, rather hard, non-painful, hemispherically raised lump on the flexor side of the right forearm, which within two weeks increased to the size of a bean, became soft in the center, and was covered with a brownish crust, after the removal of which an abscess became visible. With the softening of the primary focus a somewhat painful swelling of the right elbow and axillary glands set in. Hard, pressure-sensitive strands also formed under the skin between the abscess and the swollen elbow gland.

On 23 March 1910 the following condition was found:

On the flexor and radius side of the right forearm more or less in its center there was a not quite round, flat abscess about the size of a one-pfennig piece, surrounded by a bluish-red, somewhat wall-like border about 0.5 cm wide, here and there somewhat undermined; the base of the abscess consisted of a smooth, yellowish, area of decayed flesh, very painful to the touch, covered with a clear, thin, yellowish brown fluid and showing a few granulations as big as the head of a pin or a little bigger (Photograph no. I).



Photograph I. The inoculation sore on the fifth day after its inception, before beginning of the potassium iodide treatment; slight redness and scaling of the skin in the vicinity of the sore are remains of an iodoform dermatitis.

The lower stratum of the abscess is taken up by a hard infiltrate extending into the deeper layers of the skin, shading off into the healthy surrounding area without sharp outlines and freely movable with the skin over the tissues beneath.

Above the right condylus internus humeri a rather hard gland about the size of a bean can be felt under the skin. In the right underarm is a gland of the same consistency and about the size of a hazelnut. Both glands are slightly sensitive to pressure.

Swollen lymph glands are not visible externally. Subjectively, however, when the arm is extended a slight pain is felt running from the abscess to the swollen gland in the elbow. Upon close examination two hard strands can be found deep in the skin, connecting the abscess with the elbow gland, one in a straight line, the other in a slight curve with the convexity toward the ulnar side of the forearm. Both strands exhibit numerous nodular inspissations from the size of a lentil to that of a pea, in some cases in a row like a string of beads; some of these raise the skin visibly.

Over the swollen glands and over the inflamed lymph vessels the skin is freely movable and not discolored.

The general state of health is not affected in any way.

The clinical picture showed nothing specifically characteristic, and admitted of the following interpretations:

Apart from an abscess formation in connection with an ordinary pus coccus infection, a prominent possibility was ulcus molle; the acute course, the shape and boundary of the ulcer, the character of the floor of the ulcer, and most

especially the simultaneous occurrence of a painful swelling of the corresponding lymph glands were clinically completely consistent with ulcus molle. Although examination of the pus and granulations for streptobacilli gave a completely negative result, the ulcer was cauterized with carbolic acid and sprinkled with iodoform. This therapy had no considerable effect, and had to be broken off again immediately because of a vesicular dermatitis that ensued.

There was also some question of a tuberculous ulcer, possibly originating through inoculation. Repeated examinations for tuberculosis bacilli, which are usually easy to find in acute cases of inoculation tuberculosis of the skin, turned out negative.

For safety's sake the serous secretion was also tested for syphilis spirochetes, although the ulcer showed no similarity to a primary effect and no secondary or tertiary luetic could have been present because of lack of a prior luetic infection.

While chronic glanders of the skin can produce symptoms such as were present here, it could be ruled out in advance, because I had not come into contact with material containing glanders bacilli.

Finally a sporotrichum infection was thought of.

On 23 March at the suggestion of Chief Physician Dr. Tomasczewski serous secretion and tissue scraped from the floor and from the edge of the ulcer were transferred to five tubes of Sabouraud's maltose agar. Six days later a colony of Sporotrichum beurmanni began to grow uncontaminated in one tube; eight days after the transfer a sprotrichum colony also became visible in a second tube, alongside several diplococcus colonies. The rest of the tubes remained sterile.

On 31 March nine more maltose agar tubes were prepared with the serous secretion of the ulcer. On 3 April sporotrichum colonies began to grow in a pure state in all the tubes.

After another inoculation done on 4 April with the same kind of material numerous colonies of Sporotrichum beurmanni began to grow in a pure state on the evening of 6 April, or in other words after only two days.

That the cultures at first grew so scantily and slowly and later luxuriantly and increasingly faster must be attributed to the fact that a short time before the first inoculation of the secretion the ulcer had been sprinkled with iodoform powder and the fungus thereby temporarily inhibited in its development.

When the diagnosis of sporotrichosis appeared confirmed by the first culture, the serous secretion of the ulcer was repeatedly examined directly under the microscope for fungus

elements. For this purpose smear preparations were made, fixed in absolute alcohol, and stained in some cases by the ordinary Gram method and in others by the modification given by Much.

In all these preparations, besides diplococci and streptococci there are to be found singly or in small groups oval or irregularly roundish or angularly outlined dark violet, diffuse, grainy, or only marginally stained spore-like bodies (formes globuleuses?) and also fine, light-refracting, slightly curved or more or less strongly arched forms, here and there putting out lateral branches shorter to varying extents, much inferior in width to the size of the spore-like bodies and also to the cocci.

They show an irregular coloration, dark violet parts alternating with pale blue, barely visible ones at more or less regular intervals. The stained parts constitute forms of varying size and multifarious shape; sometimes they are round, sometimes oval, conical, or more angular in outline.

These threads are faintly reminiscent of Much's granular form of the tuberculosis bacillus, but without being identical with it; for repeated examinations of the smear preparations for tuberculosis bacilli with Ziehl-Neelsen staining came out negative.

On the other hand the threads do coincide morphologically and tinctorially with the forms first found in tissue by G. Arndt, specificallyin abscess of the testicle and in necrotic parts of testicles and lymph glands of sporotrichotic rats and which he interprets as mycelium threads of Sporotrichum beurmanni. (See also Figures 1 and 2 under experiments with animals.)

I should like to call particular attention to this finding, because it has not been possible before to detect elements of Sporotrichum beurmanni with certainty in man, either in smears of sporotrichotic material or in the tissue.

In further confirmation of the diagnosis I also repeatedly examined my blood serum for its capacity both for spore agglutination and for complement fixation, keeping to the recommendations of F. Widal, P. Abrami, E. Joltrain, Et. Brissaud, and A. Weill [see Note].

[Note] "Mycotic, etc., Serodiagnosis," Annales de l'Institut Pasteur (Annals of the Pasteur Institute), Vol 24, 1910, No 1.

The first examination was undertaken on 4 April, that is four days after the specific therapy was begun, and at a time when the clinical symptoms were still fully developed.

The complement fixation experiment gave no results that could be evaluated, as two control experiments also showed pronounced deflection of complement. For the control experiments

sera were used which were obtained from two persons certainly unaffected by mycotic disease, one of whom had given a positive and the other a negative Wassermann reaction.

In setting up the experiment I deviated from the custom of the French authors to the extent that I used not the whole culture suspension, containing threads and conidia, but an extract of it, in order to obtain as homogeneous an extract as possible, and perhaps the source of error is to be sought in this modification, assuming that the other components used in the experiment did not fail.

The test of spore agglutination undertaken on the same day gave a titre of 1:80. The spore suspension used was prepared from a culture six weeks old and not killed, and was diluted until it contained about 150 spores in the microscopic field of vision with ocular 4, objective 6 (E. Leitz). The agglutination was observed under the microscope during the first two hours after the start of the experiment. The agglutinating effect of the serum showed up within the first quarter hour in the 1:20 dilution and at the end of the first hour in the 1:40 dilution. By the end of the second hour a definite agglutination had taken place even in the 1:80 dilution; the suspension contained, besides isolated elements, numerous clumps, varying in size, of spores sticking together. The control serum, which came from a non-mycotic patient and showed no Wassermann reaction, agglutinated the same spore suspension within the first two hours only in a dilution of 1:20, the agglutination not manifesting itself until five quarter-hours after the beginning of the experiment.

In the pure spore emulsion not mixed with serum no trace of agglutination was found.

On 28 April both experiments were repeated. The ulcer at that time had been filled with epithelized granulations for eight days, and the other clinical symptoms had long since disappeared without a trace.

The complement fixation experiment, in which this time the whole suspension from a three-week-old culture was used, containing threads and spores, gave a pronounced inhibition and the control a complete solution of the sheep's blood corpuscles. (The Wassermann test done on my blood the same day by Mr. C. Hoffmann came out negative.)

On the other hand in two tests undertaken for the purpose the serum this time did not agglutinate the spores. For one of these tests the spore suspension was prepared from cultures eight weeks old and not killed, for the other from cultures six weeks old and formalinized.

On 1 May both experiments were finally run the third time, and this time neither agglutination nor complement fixation took place.

One thing that shows up from these experiments is that the serum still possessed the property of complement deflection at a time when it proved to be no longer capable of agglutination.

Treatment and Further Course

When the diagnosis of sporotrichosis appeared confirmed by the starting of the first cultures, I took 0.2 g of calcium iodide internally three times on the first day, and because of a severe iodide catarrh, 2 g of saiodin the next day and 2 and later 3 g a day for 1 1/2 months thereafter. The ulcer was only dressed with a sterile bandage. During the very first days the swelling and painfulness of the regional lymph glands and lymph vessels yielded. Only a few of the lymphangitic nodules could still be felt for a considerable time, about two weeks.

The ulcer itself healed relatively slowly.

Photograph no. II shows the ulcer five days after beginning of the calcium iodide treatment. A tongue of granulation tissue extends into the ulcer from the edge. But along with this sign of incipient healing a slight aggravation could be

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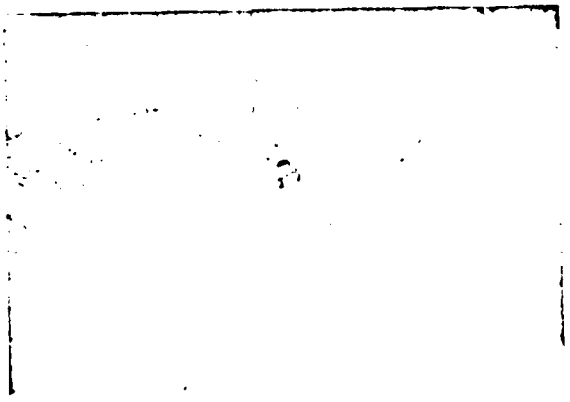

Photograph II. The same ulcer five days after beginning of the calcium iodide treatment, whose specific effect is clearly brought out here. A tongue of granulation tissue extends into the ulcer from the edge. In comparison with the first picture, at the same time, a greater depth of the floor of the ulcer can be discerned; this became evident after only a one-day interruption of the calcium iodide treatment.

discerned on the day the photograph was taken; this manifested itself in greater depth of the floor and greater serration of the edge, and set in after only a one-day interruption of the saiodin treatment.

On 22 April 1910 the ulcer was completely filled with epithelized granulations. Under pressure, however, drops of whitish-yellow pus oozed out at several points from very small, almost imperceptible openings. This pus contained numerous Gram-positive cocci, singly, in pairs, and grouped together in little chains or clumps, but no formations suggesting fungus elements. Nor were any sporotrichum colonies obtained by inoculating it in Sabouraud maltose agar tubes, but only cocci colonies.

By the beginning of May the ulcer was completely healed.

Animal Experiments (on Two Male Rats) with Material Obtained from the Process Described Above

Rat I

On 8 April a black and white rat was injected intraperitoneally with three sporotrichum colonies from the size of a pea to the size of a bean, two weeks old and in suspension in a physiological solution of common salt.

On 16 April the animal was again injected intraperitoneally with a two-weeks-old culture.

Immediately after this inoculation the rat died, as was found in dissection, from intraabdominal bleeding. Very probably the culture had been injected into the vena cava inferior or into one of its branches, for in the later histological examination of the lung, besides numerous gonidia, branched fungus fibers forming here and there a more or less dense mycelium network were found in almost all the arteries of the lung in an abundance and an unambiguous form not observed in the organs of any other experimental animal to date.

Although the first inoculation had occurred only eight days before, considerable changes were already discernible upon dissection.

The lymph glands of the gastrohepatic omentum, of the gastrocolic and gastrolienal ligaments, were swollen to the size of peas, and were of a hard consistency and smooth surface. In the gastrocolic ligament they formed conglomerates of varying size which were lying like a hood on the greater curvature of the stomach in the form of hard, lumpy tumors. When cut through they usually showed a gray-red, smooth, moist surface of section. One lymph gland was abscessed in the center; another contained in its center a cavity as large as the head of a pin and filled with yellow, friable masses.

The other organs, in particular the testicles and epidi-

dymes, showed no macroscopically discernible changes.

Sabouraud maltose agar tubes were inoculated with the tissue fluids of all the organs, with blood taken from the heart, with the pus and the friable masses of the lymph glands, and with the urine. On 20 April numerous sporotrichum colonies started up in the tubes inoculated with blood from the heart, with tissue fluids from lung, liver, kidney, spleen, and from the globus major of the epididymis. On 21 April sporotrichum cultures from the pus and the friable masses of the mesenteric glands. The other tubes (testicles, globus minor of the epididymis) remained sterile. A mold fungus developed in the urine test tube.

Smear preparations were made from the pus from the cavities of the glands and the necrotic masses; they were fixed in absolute alcohol and stained by Gram's method.

In the pus numerous sharply defined round, oval, fusiform, and more angular dark blue forms were found, some of which exhibited small round or conical bud-like appendages and a few more or less long, fine, or somewhat thicker, here and there branched threads, in which stained and unstained parts alternated almost regularly. The stained parts stand out sharply with their dark-blue color from the unstained, are of varying size and sometimes round, sometimes oval, sometimes angular shape. The pale parts of the threads are bounded by fine, pale blue, barely visible outlines; with contrast staining with safranine they take on a dull red color.

These forms are found more beautifully and definitely developed and also considerably more numerous in the smears from the friable masses. Numerous usually single but sometimes paired elongated bodies, slightly rounded or square at the ends, with diffuse violet staining, are also seen, and here and there larger round formations of irregularly grainy staining (see Figure 2). Since sporotricha can be grown in pure culture from the friable masses and the pus, the above-described formations may be assumed to be elements of Sporotrichum beurmanni, i.e. mycelia and spores.

Parts of the lung, liver, and spleen, half a kidney, one testicle, and several inflammationally altered peritoneal lymph glands were fixed and hardened in ascending percentages of alcohol, embedded in paraffin, and cut in serial sections. These were stained by various methods (van Gieson, Pappenheim, Gram, elastic fiber staining).

The lung was found to be permeated with peribronchial, more or less extensive infiltrates made up of proliferated connective-tissue cells, mononuclear round cells, and numerous plasma cells of the Marschalkó type.

The liver shows only slight small-celled infiltration in the immediate vicinity of the branches of the portal vein.

The principal changes are found in the glands. Nothing is to be seen of the actual glandular tissue. In its place is a diffuse infiltration composed of mononuclear round cells, epithelioid cells, numerous irregularly scattered giant cells, and scattered plasma cells, showing numerous microscopic and somewhat larger abscess formations. The epithelioid cells in some places form tubercular nodules, sometimes containing giant cells; they are surrounded by a zone consisting of proliferated connective-tissue cells and round cells.

The abscesses, which here and there possess a capsule of concentric layers of connective tissue, are largely composed of well-preserved leucocytes with lobate nuclei, though some exhibit necrotic portions of varying size, situated more toward the center.

One of these necrotic portions in a fairly large abscess when Gram stained shows numerous scattered dark violet bodies of irregular curved outline, which are for the most part surrounded by a fairly wide pale areola, while the central irregularly stained part possesses a granulated appearance.

We obviously have here the "formes globuleuses" of the French authors, which are to be interpreted as spores.

In the immediate vicinity of this necrotic spot was found a second, less extensive necrosis, which contained the above-described threadlike forms, probably corresponding to mycelia, in fairly abundant quantity (see Figure 1).

Rat II

On 8 April 0.2 g of the clear, serous secretion of the ulcer was injected intraperitoneally into a white rat six months old.

On 23 April a few drops of blood were taken from the rat under sterile conditions and transferred to maltose agar. In the inoculated tube yeast fungi and coccus colonies grew, but no sporotricha.

On 7 May 1910 the rat, apparently healthy, was bled to death by opening the carotid under light alcohol narcosis.

Examination of the serum for sporoagglutination and for complement fixation turned out negative.

Dissection. -- In the mesenterium of the large intestine there were several moderately hard glands, from the size of a lentil to the size of a bean, which showed a grayish red, moist, smooth surface of section.

No pathological changes could be observed macroscopically in the other organs.

Blood and tissue fluid from lung, liver, kidney, spleen,

testicles, epididymes, and mesenteric glands were inoculated on maltose agar (Sabouraud).

On 12 May colonies of Sporotrichum beurmanni began to grow in a pure state in the tubes inoculated with blood and parenchymatous fluid from the epididymes. The other tubes remained sterile.

Histological changes were found only in the lung; they completely corresponded to those found in the lung of rat I.

The normal structure can be discerned in the swollen mesenteric glands, but they contain, though only here and there, Gram-positive formations of varying form, -- firstly, straight or more or less curved threads, square or slightly rounded at the ends, which are stained intensely dark violet, either irregularly after the fashion of the threads already several times described (see Figure 4) or diffusely, and in the latter case exhibiting shallow or deeper indentations on the sides (see Figure 3); secondly, square-oval, intensely stained formations situated in pairs, which are similar to those found in the friable lymph gland masses of rat I (see Figure 3); and lastly, thick rods tapered toward the ends, with pale ends and irregularly stained light to dark blue center, which are reminiscent of de Beurmann and Gougerot's "formes mycéliennes courtes," if not in fact identical with them (see Figure 5).

If all these forms, as perhaps we are entitled to assume, are elements of Sporotrichum beurmanni, they constitute a further illustration of the multiplicity of forms of this fungus in animal tissue.

With regard to the literature I refer the reader to the work of G. Arndt cited above, which contains in its appendix a detailed list of the works published thus far on sporotrichosis.

Explanation of the Figures

Figure 1. -- Necrotic portion of an abscess of a mesenteric gland of rat I. Gram-Weigert staining, pre-staining with lithium carmine. Zeiss ocular 4, oil immersion. From the red ground, consisting for the most part of disintegrated cells, fine, more or less curved threads stand out, putting out short lateral branches here and there; in their protoplasm dark violet stained, irregularly outlined parts alternate with pale parts at more or less regular intervals.

These forms have already been found in animal tissue by G. Arndt and interpreted as mycelium fibers of Sporotrichum beurmanni.

Figure 2. -- Smear preparation from friable masses found in the cavity of a mesenteric gland of rat I. Gram staining without constrast staining. Zeiss ocular 4, oil immersion.

The field of vision contains a great number of the irregularly stained threads of varying thickness just described, alongside intensely and diffusely dark violet stained, quite irregularly shaped bodies sometimes occurring in pairs, and quite scattered larger round formations of irregularly grainy staining (spores?).

Figures 3, 4, and 5. -- Parts of an enlarged mesenteric gland of rat II, which nevertheless still shows normal structure. (Gram, staining with lithium carmine.) They show Gram-positive formations of varying shape, which correspond with great probability to elements of Sporotrichum beurmanni. Figure 3 in addition to a pair of the irregularly shaped bodies just described (Figure 2) contains a diffusely stained thread exhibiting shallow indentations, Figure 4 a quite irregularly stained threadlike form, and lastly Figure 5 an irregularly stained rod, tapered at the ends, which is reminiscent of de Beurmann's "forme courte mycélienne."